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ORIGINAL ARTICLE – MELANOMAS

Use of S-100B to Evaluate Therapy Effects during Bevacizumab Induction Treatment in AJCC Stage III Melanoma

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ABSTRACT

Aim. To investigate the feasibility of using bevacizumab to improve the survival of American Joint Committee on Cancer (AJCC) stage III melanoma patients, we investigated how a single bevacizumab treatment affected nodal disease and a panel of biomarkers in clinically fluorodeoxyglucose positron emission tomography (FDG-PET)/computed tomography (CT)-staged, stage III melanoma patients, prior to therapeutic lymph node dissection (TLND).

Methods. Four weeks before TLND, nine patients (median age 50, range 28.8–62.1 years; two male, seven female) with palpable lymph node metastases received 7.5 mg/kg bevacizumab. Before and after this treatment, all patients were assessed by measurements of the maximum standardized uptake value (SUVmax) by FDG-PET scan, and serum S-100B and lactate dehydrogenase (LDH). After TLND, the dissection specimen was analyzed for number of removed lymph nodes, number of metastatic lymph nodes, and tumor necrosis.

Results. Median follow-up was 15.5 (2.2–32.9) months. Histopathological analysis revealed tumor necrosis in six patients, of whom five had an S-100B decline and one had an unchanged S-100B level after bevacizumab. The other three patients showed an S-100B increase and no necrosis. Tumor necrosis was correlated with S-100B decrease ($P = 0.048$). No association was found between necrosis and the markers SUVmax and LDH. No wound healing disturbances were encountered.

Conclusion. Tumor necrosis in dissection specimens was associated with declining S-100B levels, while elevated S-100B was only found in cases with no necrosis. Bevacizumab might be useful in treating AJCC stage III melanoma patients prior to TLND, and S100-B appears to be a useful marker for assessment of treatment effects.

Melanoma is an aggressive and highly metastatic disease, which can be fatal with a rapid systemic dissemination. Approximately one-third of all melanoma patients will experience disease recurrence.[1,2] While almost all organs can be involved, the most frequent target sites are the liver, bone, and the brain. Treatment results for advanced melanoma remain unsatisfactory. No systemic therapy has been demonstrated to affect overall survival, although recent studies of immunotherapy with ipilimumab and the introduction of BRAF pathway inhibitors have shown promising results.[3–5] For melanoma patients with nodal disease, therapeutic lymph node dissection (TLND), with or without adjuvant radiation, remains the only curative therapy, with 5-year survival rates of 78, 59, and 40%, respectively, for patients with AJCC stage IIIA, IIIB, and IIIC disease.[6,7] As a consequence of shortages in healthcare resources, the growing elderly population in the Western world, and the increasing incidence of cancer, the wait time for surgery at some cancer centers has lengthened to an average of 4–5 weeks. This period before TLND offers a unique opportunity to test novel induction treatments before surgery.

Tumor angiogenesis is a continuous process that allows cancer cells to grow by supplying the tumor with nutrients

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and oxygen, disposing of metabolic waste products, and providing a route for metastatic spread.[8,9] Vascular endothelial growth factor A (VEGF-A) is a key growth factor involved in the development and maintenance of tumor angiogenesis.[10] Bevacizumab, a fully humanized monoclonal antibody, binds to all VEGF isoforms with high affinity, thereby blocking ligand–receptor signaling.[11] It is currently used in patients with metastatic colon cancer, non-small-cell lung cancer, and renal cell cancer.[12–14] Bevacizumab was previously evaluated in a randomized phase II trial (BEAM trial) in metastatic melanoma, which compared the effects of the combination of carboplatin and paclitaxel, with and without bevacizumab. The addition of bevacizumab had a significant impact on progression-free survival, and some impact on overall survival, although this effect was not significant.[15]

The time spent waiting for a TLND for regional metastatic disease could be used more effectively if an induction therapy could be safely administered to reduce tumor load before surgery. S-100B and SUV are known to be of prognostic value in stage III melanoma; elevated S-100B and SUV in stage III melanoma patients can be specific indicators of disease progression.[16–22] Therefore, we hypothesized that monitoring S-100B and SUV before and after a single bevacizumab treatment might provide a "measurable reflection" of the response to this angiogenic treatment.

Here, we investigated the feasibility of using serum biomarkers, S-100B and LDH, and the standardized uptake value (SUV) from FDG-PET to evaluate effects of an induction treatment with a single dose of bevacizumab in stage IIIB/C melanoma patients, prior to TLND. We assessed the perioperative changes in biomarker levels following bevacizumab treatment, as well as the induction of tumor necrosis based on final histopathology of the resected lymph nodes.

PATIENTS AND METHODS

Patients

All consecutive melanoma patients presenting with palpable and cytology-proven lymph node metastases (AJCC IIIB/C) at the University Medical Centre Groningen (UMCG) between January and July 2008 were evaluated with FDG-PET and spiral CT in addition to routine staging, which included a complete medical history, physical examination, and blood chemistry profile. If the FDG-PET and CT were negative for distant metastases, patients were offered participation in the bevacizumab trial bridging the waiting time between diagnosis and TLND. Eligible patients were $\geq$18 years of age with AJCC stage IIIb melanoma and World Health Organization (WHO) performance status of 0–2. Exclusion criteria included history of radiotherapy to the involved lymph node basin, major surgery within 28 days of start of the study, administration of any investigational drug within 30 days before start of the study, and clinical evidence of brain metastases. Patients who presented with positive sentinel lymph node biopsy, local recurrence, and/or in-transit disease were also excluded from this study. Examined data included patient demographics, primary tumor characteristics, date and type of operation, and status at last follow-up. Patients with nodal metastases of $\geq$3 cm and/or $\geq$3 positive lymph nodes and/or extranodal (EN) disease received adjuvant radiotherapy (20 $\times$ 2.4 Gy).

This study was approved by the local medical ethics committee, and written informed consent was obtained from all participants. The study was registered under trial number NTR1941 and was organized as a corollary study in concurrence with another feasibility study investigating the presence of VEGF in melanoma lesions by VEGF-single-photon emission computed tomography (SPECT) with ^{111}In-bevacizumab.[23]

Study Design

On day 0, all selected patients were evaluated with a FDG-PET scan, and on day 7, they received a single dose of 7.5 mg/kg bevacizumab intravenously. On day 40, the patients underwent a second FDG-PET scan, after which TLND was performed on day 42 (5 weeks after bevacizumab). S-100B and LDH levels were measured on days 0, 41, and 43 (first visit to outpatient clinic, 1 day preoperatively, and 1 day postoperatively). After lymph node dissection, the pathological results were analyzed.

S-100B

Concentrations of serum S-100B were measured using the Liason Sangtec 100 immunoassay on an Advantage (Nichols). The half-life of S-100B is estimated to be about 30 min.[24] Levels were calculated using a calibration curve and checked against internal standards with known concentrations of S-100B. In this assay, concentrations were considered normal if they were below a cutoff point of 0.15 μg/l. Concentrations above this range were considered to be elevated. For all patients, the change in S-100B concentration from day 0 to day 41 was determined.

LDH

LDH was routinely analyzed by enzymatic activity measurement with a Roche Modular analyzer (Hitachi). LDH values <250 U/l were considered to be normal.

Standardized Uptake Value (SUV)

To assess the degree of FDG accumulation before and after bevacizumab treatment, the maximum standardized uptake value (SUVmax) was calculated from a FDG-PET scan. The SUV depends on the amount of injected radioactivity, the patient's weight, and the calibration factor of the camera, and it is calculated according to the following formula: SUVmax = radioactivity concentration in tissue (Bq/kg)/(injected dose [Bq]/patient weight [kg]). If multiple metastases were present in the lymph node basin, the lesion with the most intense uptake was chosen. The cutoff point for SUV is data driven and therefore depends on the patient group; there is no consensus in the literature regarding a cutoff point for SUV.[25–27] In the present study, any decrease/increase of SUVmax was recorded simply as the level of decrease/increase.

Surgery

The therapeutic axillary lymph node dissection included a level III resection with resection of the pectoralis minor muscle. The groin lymph node dissection included a superficial (inguinal) and deep (iliac-obturatory) groin dissection. The latter procedure was performed as extensively described by Baas et al.[28] Perioperative surgical complications, number of harvested lymph nodes, number of metastatic lymph nodes, maximum metastasis diameter, and degree of tumor necrosis were scored. The pathological results after lymph node dissection were analyzed by a pathologist.

Statistics

Fisher's exact test was used to assess associations between the SUV, S-100B and LDH levels, and pathology data.

RESULTS

Patient Characteristics

Table 1 presents the characteristics of the nine AJCC stage III melanoma patients, who included two males and seven females, with median age of 50 (range 28.8–62.1) years. Median follow-up time was 15.5 (range 2.2–32.9) months. In these nine patients, metastatic lymph nodes were detected with spiral CT and FDG-PET imaging. After TLND, the resection specimens displayed a median of 16 nodes (range 8–29). No perioperative or wound healing disturbances were encountered.

TABLE 1 Characteristics of the primary melanoma and localization of recurrence of the nine patients in the study

	Number	%
Sex		
Male	2	22.2
Female	7	77.8
Age, years, median (range)		
50.0 (28.8–62.1)	–	–
Localization of primary melanoma		
Trunk	5	55.6
Upper extremities	0	0
Lower extremities	3	33.3
Unknown	1	11.1
Clark level		
II	1	11.1
III	2	22.2
IV	5	55.6
Unknown primary	1	11.1
Breslow thickness (mm)		
≤1.0	4	44.4
>1.0	4	44.4
Unknown primary	1	11.1
Mitotic index (n)		
≤5	4	44.4
≥6	4	44.4
Unknown primary	1	11.2
Ulceration		
Yes	3	33.3
No	5	55.6
Unknown primary	1	11.1
Localization of lymph node metastases		
Axilla	5	55.6
Groin	4	44.4

SUV and LDG

In five of the nine patients, SUVmax decreased after bevacizumab treatment. In one patient, SUVmax was not influenced, and in the remaining three patients, SUVmax increased. Between day 0 and day 41, an LDH decrease was noted in seven patients and an elevation in two patients.

S-100B and Pathology

The pathologic analysis reported tumor necrosis in six TLND specimens after bevacizumab treatment. In five of these six patients, a decrease in S-100B after bevacizumab was documented on day 41; in the remaining one patient, S-100B was unchanged but was comparatively decreased on day 43. Tumor necrosis was significantly associated

TABLE 2 Association between pathology data and changes in SUV value, S-100B, and LDH

	SUV		S-100B		LDH	
	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Size						
≤4.0 cm	3 (75)	1 (20)	2 (50)	2 (40)	1 (50)	3 (43)
>4.0 cm	1 (25)	4 (80)	2 (50)	3 (60)	1 (50)	4 (57)
Necrosis						
Yes	3 (75)	3 (60)	1 (25)[a]	5 (100)[a]	0 (0)	6 (86)
No	1 (25)	2 (40)	3 (75)	0 (0)	2 (100)	1 (14)
Ratio positive/removed nodes						
≤0.5	2 (50)	2 (40)	2 (50)	2 (40)	1 (50)	3 (43)
>0.5	2 (50)	3 (60)	2 (50)	3 (60)	1 (50)	4 (57)
Extranodal growth						
No	2 (50)	3 (60)	3 (75)	2 (40)	1 (50)	4 (57)
Yes	2 (50)	2 (40)	1 (25)	3 (60)	1 (50)	3 (43)
Total	4 (44)	5 (56)	4 (44)	5 (56)	2 (22)	7 (78)

S-100B in µg/l

Patients: $n = 9$

[a] $P < 0.05$ (Fisher's exact test)

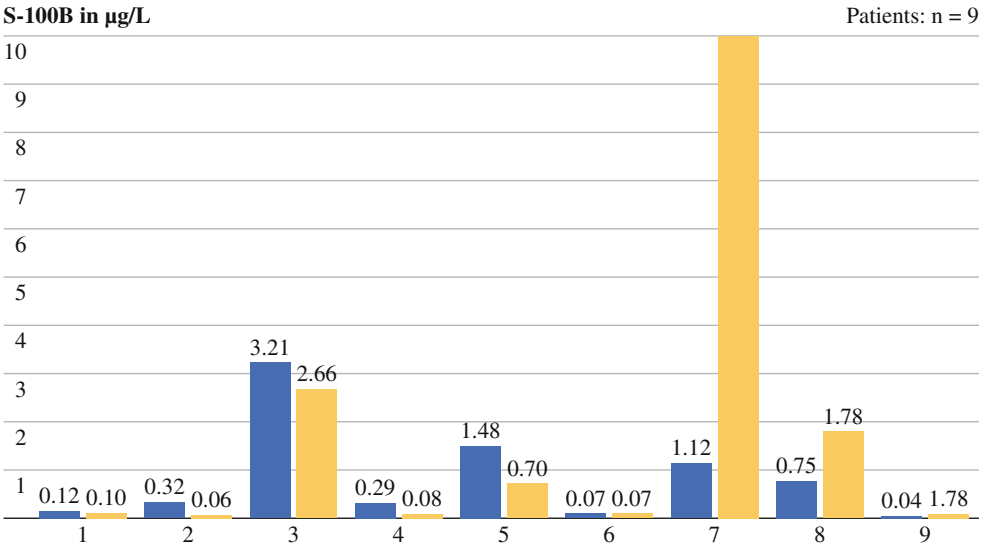

FIG. 1 S-100B before and after bevacizumab

with declining S-100B levels ($P = 0.048$). These data are presented in Table 2 and Fig. 1. No association was found between the markers SUVmax and S-100B ($P = 0.6$) or LDH, nor was a correlation found between LDH or SUVmax and necrosis (Fig. 2).

DISCUSSION

This is the first study to assess the use of biomarkers to monitor the response following an induction treatment with bevacizumab, a VEGF-specific antibody, prior to TLND in AJCC stage III melanoma patients with nodal disease. Our data suggest that neoadjuvant bevacizumab may be useful for preoperative tumor load reduction before TLND in AJCC stage IIIB/C melanoma patients.

Neoadjuvant treatment with bevacizumab might have induced tumor necrosis in the metastatic lymph nodes in six of the nine patients. In all patients with necrosis in the dissection specimen, a significant decline of serum S-100B levels was observed. In contrast, in patients whose S-100B levels continued to rise following treatment, no necrosis was found in the dissection preparations. No relationship was found between SUV and S-100B or LDH and S-100B or any of the other pathological data.

The data from this feasibility study should only be considered preliminary, and it is clear that no firm conclusions can be drawn from such a small group of samples. However, the observations certainly indicate that further investigation is warranted. The data from the present study might indicate that a single neoadjuvant bevacizumab treatment induced tumor necrosis in the tumor-bearing lymph nodes. Even more importantly, a decline of S-100B was observed in all patients who exhibited tumor necrosis after induction therapy.

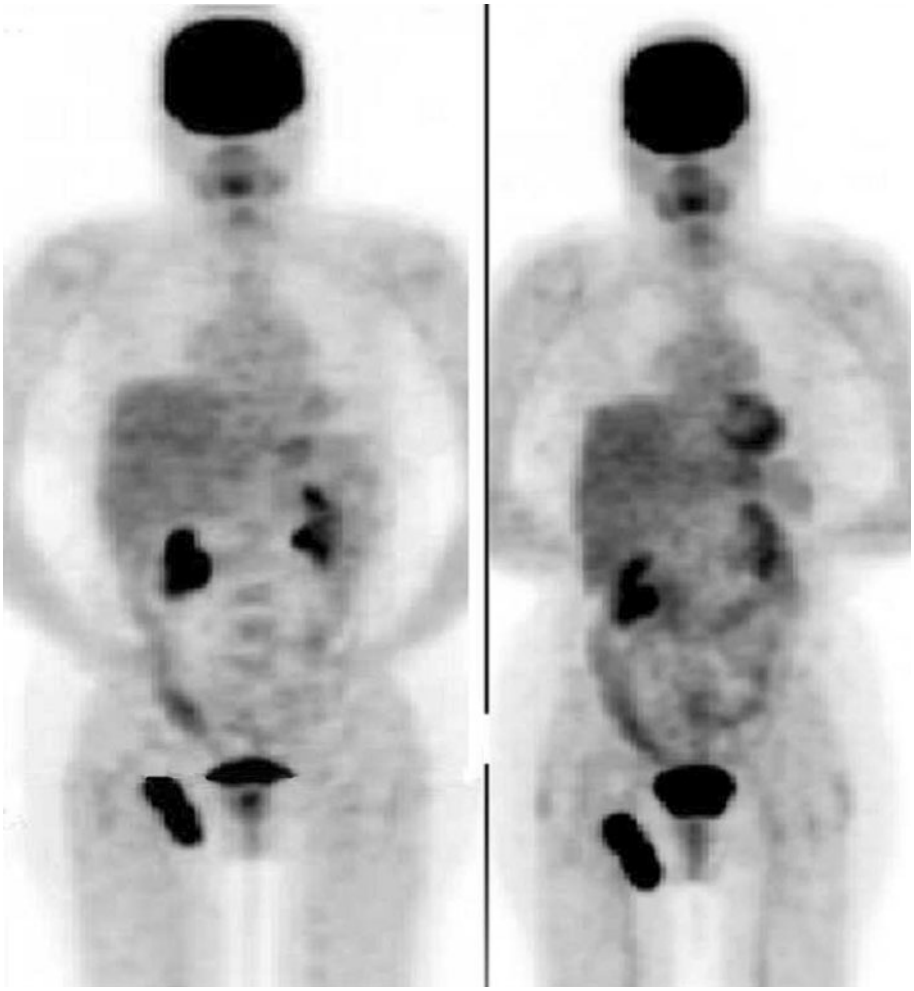

FIG. 2 Patient (61 years, female), melanoma lower extremities (Breslow 2.1 mm, ulceration). Inguinal lymph nodes metastases with a SUVmax of 9.2 first scan and 10.5 second scan

Unfortunately, there are no data available revealing how often necrosis is discovered after lymph node dissection without prior administration of any induction therapy. However, the relatively high necrosis rate within our small sample of patients and the related S-100B decline might suggest a drug-induced tumor necrosis in the lymph nodes. The associated significant decrease of tumor load might explain the S-100B decline in all six patients.

To date, the most extensively studied biomarker in melanoma is S-100B, a 21-kDa protein that was first isolated from the central nervous system in vertebrates. S-100B is located in the cytoplasm and nucleus in cells of neuroectodermal origin, e.g., melanocytes. S-100B has various intracellular functions, mainly in cytoskeleton integrity, cell cycle regulation, and apoptosis. Elevated serum S-100B occurs as the result of loss of melanoma cell integrity.[24] The mechanism by which this protein is released into circulation remains uncertain, but it is probably caused by cell damage or apoptosis. The hypothesis that elevation of S-100B should be interpreted as indicating ongoing metastatic disease has been suggested by earlier data.[16] Proportions of patients with elevated S-100B concentrations are 0–9% in stage I/II, 5–98% in stage III, and 40–100% in stage IV melanoma.[29] For clinically FDG-PET and CT-staged stage III patients, a preoperatively elevated S-100B level is correlated with decreased survival.[16–19] In stage IV, improved survival is observed for patients with low S-100B levels; increased concentrations of S-100B during systemic treatment are associated with disease progression.[30–34] Recently, Bouwhuis et al. revealed that elevation of S-100B levels throughout serial serum measurements in stage III is of very significant prognostic value, which was even stronger when combined with disease stage and number of positive lymph nodes.[35] However, elevated S-100B should not only be seen as a prognostic factor for survival and a mere reflection of disease progression alone; the presence of the protein S-100B itself induces disease progression as well. S-100B interacts with p53 and thereby downregulates its function as a tumor suppressor protein by preventing induction of apoptosis of potential melanoma cells.[36,37]

Taking these results into account, an induction therapy before lymph node dissection might interrupt the melanoma proliferation cycle in two ways: first by reducing

tumor load, and second by temporarily suppressing serum S-100B concentrations and thus preventing downregulation of tumor suppressor protein p53. Bevacizumab as an induction therapy might temporarily suppress metastatic proliferation and eventually, in combination with surgery, improve outcome.

Antiangiogenic therapy is a promising strategy in the treatment of cancer. Angiogenesis, or remodeling of an existing network of blood vessels, performs an essential role in diverse pathophysiological processes.[38,39] Because the neovascularization process that supports tumor growth may be similar to that involved in physiological wound healing, a delay in wound repair has been a concern. This may be one of the reasons that bevacizumab has not been widely used as induction therapy before major surgical cancer treatment.[40] None of the patients in our study showed any would healing disturbances during the course of the study.

The ongoing AVAST-M trial is the only known randomized trial evaluating the VEGF inhibitor, bevacizumab, for adjuvant therapy following resection of AJCC stage IIB ($T4aN_0M_0$), IIC ($T4bN_0M_0$), and III ($TxN_{1-3}M_0$) cutaneous melanoma. Many randomized trials of adjuvant therapy have been performed in patients after resection of melanoma, but to date, no treatment has convincingly improved overall survival. The AVAST-M trial is a Cancer Research UK-funded, National Cancer Research Network (NCRN) trial in which patients with previously resected melanoma at high risk of disease recurrence will be randomized to receive either standard observation or bevacizumab administration for 1 year.[41]

In one of the few examples in which induction therapy effects were monitored by biomarkers, Willet et al. performed a phase I–II trial to test the efficacy of adding bevacizumab to standard chemoradiotherapy as an induction therapy before rectal surgery. The phase II clinical trial involved administration of neoadjuvant bevacizumab, in combination with 5-fluorouracil (5-FU) and radiation therapy. The response of one patient's tumor to this regimen with bevacizumab was dramatic, with no residual cancer in the surgical specimen. More surprisingly, plasma carcinoembryonic antigen (CEA) dropped substantially from a pretreatment value of 122.8–63.6 ng/ml at 12 days after the first bevacizumab infusion, and declined to 4.9 ng/ml at 1 month after completion of neoadjuvant therapy before surgery.[42]

In addition to S-100B, we also monitored SUVmax and LDH levels in the patients in this study, but did not find any significant correlation between these values and S-100B or pathology findings. In an earlier study, no relation was found between the prognostic markers S-100B and SUV in melanoma patients with palpable lymph nodes.[43] Evidence concerning FDG uptake suggests that intensity of uptake does not correlate with lymph node tumor burden, but more with biological aggressiveness.[44] Serum lactate dehydrogenase (LDH) has high specificity for melanoma but low sensitivity; it is an independent prognostic factor in stage IV melanoma.[16] Taking these facts into consideration, it is not surprising that we observed a lack of correlation of these markers with tumor response in this study.

In conclusion, this feasibility study has shown that induction treatment with bevacizumab in stage III melanoma 4 weeks prior to surgery may be related to induction of tumor necrosis without any observed incidence of wound healing disturbance. More importantly, when tumor necrosis was found in the dissection preparation, a 100% decline of S-100B was noted. Correspondingly, absence of tumor necrosis was correlated with ongoing S-100B elevation. The ability to target therapy towards well-selected subgroups of patients with the use of biomarkers, such as S-100B, could increase the likelihood of benefit and might improve therapeutic outcomes for future melanoma treatments.[45] Induction treatment with bevacizumab followed by therapeutic lymph node dissection could be a potentially successful combined treatment and might improve locoregional control, and therefore disease-free survival, for lymphogenic disseminated melanoma patients.

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